

THE EFFECTS OF BICARBONATE AND MINERAL SURFACES ON URANIUM
IMMOBILIZATION UNDER ANAEROBIC CONDITIONS

BY

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THESIS

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Abstract

For four decades, from 1940 through 1980, the U.S. Department of Energy (DoE) extensively mined and processed uranium at various sites. As a result, widespread uranium contamination exists in subsurface sediments and aquifers. In subsurface environments, uranium primarily exists as U(VI) or U(IV), oxidized and reduced species, respectively. U(VI) is highly soluble and toxic, U(IV), while relatively toxic, is insoluble which greatly reduces its exposure pathways.

We seek to examine the role of ferric iron on U(VI) reduction by adsorbing U(VI) onto ferric and non-ferric mineral surfaces in the presence of a reductant. Further, we seek to understand the role that NaHCO_3 , a natural groundwater buffer, has in the reductive geochemical transformations of U(VI) adsorbed on ferric and non-ferric mineral surfaces. Bench top studies were performed using $100 \mu\text{M}$ U(VI) and the reductant AHQDS, in the presence and absence of Fe-Gel (amorphous ferric oxyhydroxide) and $\gamma\text{-Al}_2\text{O}_3$. In the presence of a HEPES buffer at pH 8, results demonstrate direct homogeneous reduction in several hours in the absence of Fe-Gel or $\gamma\text{-Al}_2\text{O}_3$, and reduction within a 48-hour period in the presence Fe-Gel or $\gamma\text{-Al}_2\text{O}_3$. While adsorbed to both ferric and non-ferric mineral surfaces, U(VI) reduction is inhibited. U(VI) reduction in the presence of NaHCO_3 buffer also inhibits U(VI) reduction.

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Introduction

Uranium has been identified as a major groundwater contaminant at various United States Department of Energy sites, as well as locations where uranium was mined and processed (Barnett, Jardine et al. 2000; Finneran, Anderson et al. 2002; Wazne, Korfiatis et al. 2003; Zhou and Gu 2005; Boonchayaanant, Nayak et al. 2009). Oxidized uranium, U(VI), is readily soluble and poses threats to down gradient receptors. Reduced uranium, U(IV), is a relatively insoluble solid under anoxic conditions, which greatly reduces the risk of exposure. U(VI) typically exists as the uranyl cation, UO_2^{2+} , or as a uranyl complex. U(IV) in reducing conditions exists as the solid phase compound, UO_2 , called uraninite. U(VI) has been shown to interact strongly with solid phases; therefore, the fate and transport of uranium in groundwater is strongly influenced by U(VI)-particle interactions (Barnett, Jardine et al. 2000). Further complicating the fate and transport of U(VI) is the concentration and presence of dissolved carbonates, which act as the natural buffer for most surface waters and groundwater. U(VI) is thermodynamically favored to form stable complexes with carbonate anions (Guillaumont 2003). U(VI)-carbonate complexes, such as $\text{UO}_2(\text{CO}_3)_3^{4-}$ and $\text{UO}_2(\text{CO}_3)_2^{2-}$, have poor interactions with soils and solid mineral phases; therefore, these stable complexes are highly mobile in soils and groundwater (Zhou and Gu 2005).

Recent efforts to facilitate the remediation of uranium have focused on using in situ bioremediation to reduce soluble U(VI) to insoluble U(IV), which is significantly less expensive than pump-and-treat technologies and eliminates the need for the disposal of a hazardous waste (Lovley, Phillips et al. 1991; Finneran, Anderson et al. 2002; Boonchayaanant, Nayak et al. 2009). Extra-cellular electron shuttles are being explored as an alternative to direct biological reduction; they promote abiotic reduction of U(VI) to U(IV) (Jeon, Dempsey et al. 2005). Humic substances are excellent extra-cellular electron shuttles; they contain quinone functional groups that cycle between oxidation states to donate and accept electrons (Liger, Charlet et al. 1999; Fredrickson, Zachara et al. 2000; Jeon, Dempsey et al. 2005; Wang, Wagnon et al. 2008). There is strong evidence that the rate of U(VI) reduction to U(IV) is affected by sorption to redox active mineral surfaces. For example, U(VI) was reductively precipitated by Fe(II) in the presence of pyrite (Liger, Charlet et al. 1999). However there are a lack of studies demonstrating U(VI) reactivity on

surfaces which are not iron based, and a lack of studies demonstrating the effect of carbonate species on U(VI) reactivity with abiotic reductants.

The purpose of this project is to study the effects that different mineral surfaces and carbonates have on U(VI) reduction by the extracellular electron shuttle anthrahydroquinone-2,6-disulfonate (AHQDS). Specifically, this project seeks to investigate the roles that redox-active and non-redox active mineral surfaces play in reduction of U(VI) adsorbed to mineral surfaces. The project also seeks to understand what role dissolved carbonates, a natural water buffer, play in the reduction of U(VI) adsorbed on minerals surfaces. Two hypotheses will be tested. First, redox-active mineral surfaces are responsible for the reduction of adsorbed U(VI) via a surface catalysis effect. Second, in the presence of a carbonate buffered system, U(VI) reduction will be inhibited due to the strong complexation effects of carbonates with the uranyl cation (UO_2^{2+}).

Background

Sorption of Uranium on Solids

Uranium sorption to mineral surfaces is a highly important mechanism that retards the mobility of the metal in subsurface environments (Tang and Reeder 2009). Nuclear and radioactive waste depository safety is evaluated based on the sorption of radionuclides to natural and engineered barriers (Lefèvre, Noinville et al. 2006). Soils and sediments are highly heterogeneous in nature, containing many different mineral phases, organic materials, and humic substances. Iron, specifically Fe(III) oxides, are widespread in natural sediments and in certain cases represent the most abundant electron accepting compounds by mass in some aquifer sediments (Fredrickson, Zachara et al. 2000). Studies have shown that oxide minerals, in particular ferric oxides, are also good sorbents of U(VI) (Liger, Charlet et al. 1999; Lefèvre, Noinville et al. 2006). At circumneutral pH, γ -alumina has been shown to be a good adsorbent of U(VI) (Tang and Reeder 2009). For uranium remediation to be effective in the long term, the approach taken must address the mobile, adsorbed, and precipitate phases of U(VI), of which the latter two are a potential long term source of U(VI) contamination (Fredrickson, Zachara et al. 2000) if not immobilized in a stable form.

Surface Effects on Redox Reactions

The redox chemistry of mineral surfaces plays an important role in contaminant destruction and immobilization in subsurface environments. Mineral surfaces consisting of Fe(II) can contribute to the reduction of both organic and metal contaminants, including U(VI) (Scherer, Balko et al. 1999; Boonchayaanant, Nayak et al. 2009). Microbial metabolism yields reduced products such as ferrous iron, which can abiotically reduce U(VI) to U(IV) (Boonchayaanant, Nayak et al. 2009). In anoxic, subsurface environments, the Fe(III)-Fe(II) redox cycle is driven by microbial respiration, which generates a continuous flux of Fe(II) ions that can complex to mineral surfaces and promote the natural attenuation of many classes of groundwater contaminants (Williams and Scherer 2004). Mineral surfaces have been shown to dramatically increase the reduction rates of contaminants by Fe(II) due to Fe(II)-mineral surface complexation effects (Liger, Charlet et al. 1999; Williams and Scherer 2004). Much evidence suggests that the iron redox cycle and uranium ore deposit formations share an intimate link; specifically, U(VI) was reductively precipitated by Fe(II) in the presence of pyrite (Liger, Charlet et al. 1999). The Fe(II) mediated abiotic reduction of U(VI) sorbed onto Fe(III) oxides has been shown to occur at circumneutral pH

(Jeon, Dempsey et al. 2005). Studies performed by Liger et. al. (Liger, Charlet et al. 1999) demonstrate that adsorption of reactants to mineral surfaces catalyze the abiotic reduction of uranyl by Fe(II).

Redox Reactions Mediated by Electron Shuttling Compounds

Subsurface sediments and soils contain natural organic matter (NOM), which are non-living organic compounds generated by the decomposition of organic materials and microbial by-products. NOM is believed to contain a significant number of quinone moieties in its molecular structure (Uchimiya and Stone 2006). Studies employing nuclear magnetic resonance (NMR), electron spin resonance (ESR), and cyclic voltammetry suggest that the primary redox active group in NOM and humic materials are these quinone moieties (Nevin and Lovley 2000; Uchimiya and Stone 2009). As a result, several biogeochemical processes, such as iron redox cycles, are believed to involve naturally occurring quinones (Nevin and Lovley 2000; Uchimiya and Stone 2006; Uchimiya and Stone 2009). Thus far, it has been shown that humic substances can serve as electron acceptors in the respiration of all evaluated Fe(III)-reducing bacteria and archaea (Nevin and Lovley 2000; Uchimiya and Stone 2009). NOM and humics, because of their redox-active nature, are important compounds in understanding the overall redox transformation of contaminants occurring in soils and sediments. The reductive degradation of contaminants via electron transfer reactions catalyzed by quinones have recently received much attention (Uchimiya and Stone 2009). Quinone moieties are reduced to hydroquinone groups via microbial respiration processes (Nevin and Lovley 2000). The hydroquinones can act as electron shuttles and abiotically reduce Fe(III) to Fe(II) (Nevin and Lovley 2000). Fe(III) oxides were reduced much faster when quinones or humics were present than absent (Nevin and Lovley 2000). Anthraquinone-2,6-disulfonate (AQDS) has been an important model quinone NOM moiety that has been extensively studied (Uchimiya and Stone 2006). U(VI) has been quantitatively reduced by the reduced form of AQDS, anthrahydroquinone-2,6-disulfonate (AHQDS), both in solution and when uranium is sorbed to iron oxide surfaces (Jeon, Dempsey et al. 2005; Wang, Wagnon et al. 2008).

Methods

Materials

The following is a list of materials and their source used in this project: NaHCO₃ Sigma-Aldrich, 99.7% purity; Na₂CO₃ Sigma-Aldrich, 99.5% purity; NH₄Cl, Sigma-Aldrich, cell culture tested; NaH₂PO₄•H₂O, Sigma-Aldrich, 98% purity; KCl 99% purity; Na₂SeO₄ 98% purity; Arsenazo III, Fluka; AQDS, Sigma-Aldrich, >98% purity; UO₂Cl₂•3H₂O, IBILabs; γ-Al₂O₃, DeGussa; Sodium Acetate, Sigma-Aldrich, cell culture tested.

Anaerobic Culture

The organism utilized in this study is *Geobacter metallireducens* (GS-15). The GS-15 pure culture was available in Dr. Kevin T. Finneran's laboratory. The GS-15 pure cultures were grown in anaerobic pressure tubes at 30°C, under a N₂/CO₂ (80:20) atmosphere, in ferric citrate media. The anaerobic pressure tubes were sealed with thick butyl rubber stoppers and crimped. The ferric citrate media was prepared with the following components (g/L unless specified otherwise): Ferric Citrate, 13.7; NaHCO₃, 2.5; NH₄Cl, 0.25; NaH₂PO₄•H₂O, 0.6; KCl, 0.1; modified Wolfe's vitamin and mineral mixtures (each 10 mL/L) from stocks and 1mL of 1mM Na₂SeO₄. The final concentrations of vitamins in the freshwater media are as follows: 20 µg biotin, 20 µg folic acid, 100 µg pyridoxine HCl, 50 µg riboflavin, 50 µg thiamine, 50 µg nicotinic acid, 50 µg pantothenic, 1 µg B-12, 50 µg p-aminobenzoic acid, 50 µg thioctic acid; the final mineral concentrations are: 15 mg NTA, 30 mg MgSO₄, 5 mg MnSO₄•H₂O, 10 mg NaCl, 1 mg FeSO₄•7H₂O, 1 mg CaCl₂•2H₂O, 1 mg CoCl₂•6H₂O, 1.3 mg ZnCl₂, 100 µg CuSO₄•5H₂O, 100 µg AlK(SO₄)₂•12H₂O, 100 µg H₃BO₃, 250 µg Na₂MoO₄, 240 µg NiCl₂•6H₂O, 250 µg Na₂WO₄•2H₂O and 189 µg Na₂SeO₄. Following media preparation and sealing of the pressure tubes, the tubes were autoclaved for 20 minutes at 120°C. The electron donor used for GS-15 growth was sodium acetate. Sodium acetate stock was prepared and stored in an anaerobic serum bottle under a N₂ atmosphere, sealed with a thick butyl rubber stopper, crimped, and autoclaved for 20 minutes at 120°C. Sodium acetate stock was amended to the ferric citrate media via aseptic and anaerobic techniques to a final concentration of 20 mM.

Preparation of the Quinone and Hydroquinone Stocks

The quinone and hydroquinone used in these studies are anthraquinone-2,6-disulfonic acid (AQDS) and anthrahydroquinone-2,6-disulfonic acid (AHQDS), respectively. 40 mM AQDS stock solutions were prepared from anthraquinone-2,6-disulfonic acid disodium salt

and buffered at pH 8 with 10 mM HEPES buffer. The stocks were stored in anaerobic serum bottles under a N₂ atmosphere, sealed with a thick butyl rubber stopper, crimped, and autoclaved for 20 minutes at 120°C. The stock bottles were wrapped in aluminum foil and stored in the dark. The hydroquinone, AHQDS, was generated via microbial reduction of 40 mM AQDS stock in 10 mM HEPES buffer. Microbial reduction was achieved via a cell suspension using GS-15 as the microbial catalyst and acetate as the electron donor. The following items were used in the cell suspension: 890mL anaerobic (80:20 N₂/CO₂) and sterile ferric citrate media, 4-500mL centrifuge tubes, 4-50mL centrifuge tubes, 1-10mL anaerobic tube (top sealed with foil), 1 blue butyl rubber stopper wrapped in foil, 10 steel cannulas wrapped in foil, 10-0.2 µm sterile Nalgene filters, 1-10mL sterile pipette, 1-50 mL sterile pipette, 1-400 mL beaker (top sealed with foil), and 350mL anaerobic (N₂) 10 mM HEPES buffer stock. All of the previous items listed were autoclaved for 20 minutes at 120°C prior to use in the cell suspension experiment. The GS-15 pure culture was grown in ferric citrate media at 30°C, 100 mL total volume, with 20 mM acetate as the electron donor. After 24 hours of growth, 10 percent total volume (100 mL in this case), of cells was transferred to the 890 mL ferric citrate media and amended with acetate to a final concentration of 20 mM acetate. The final volume of GS-15 pure culture was one liter. The one liter of pure culture was incubated at 30°C for 13 hours, which corresponds to the midpoint of the exponential log-phase growth state of GS-15 in ferric citrate utilizing acetate as the electron donor. At this time, the one liter anaerobic pure culture was opened and sterile cannula were attached to 0.2 µm sterile filters, inserted to the bottle, and a high flow rate of N₂/CO₂ (80:20) was started to keep the environment anoxic. Next, 250 mL of pure culture was distributed into the four, 500 mL, sterile centrifuge tubes. While distributing the pure culture into the centrifuge tubes, sterile cannulas attached to 0.2 µm sterile filters were inserted into the centrifuge tubes and pure culture bottle while simultaneously delivering a high flow rate of N₂/CO₂ (80:20) into the centrifuge tubes and the pure culture bottle. After pouring the 250 mL of pure culture into each of the four centrifuge tubes, the high flow of N₂/CO₂ was kept running for one minute to create an anoxic headspace. The centrifuge tubes were then sealed, placed in a centrifuge cooled to 4°C, and centrifuged at 5000g for 20 minutes. When the centrifuge cycle was completed, the tubes were removed, and the supernatant was carefully decanted making sure to not disturb the cell pellet that remained. While decanting, a high flow of N₂/CO₂ (80:20) was introduced into the centrifuge tubes to keep the cell pellet anoxic. After decanting, each cell pellet was washed and resuspended

with 35 mL of 10 mM, anaerobic, HEPES buffer. The 35 mL of cell/buffer solution was then distributed into the four, 50 mL, centrifuge tubes. A high flow of N₂/CO₂ (80:20) was kept running into both 500 mL and 50 mL centrifuge tubes during resuspension and transfer. The 50 mL centrifuge tubes were sealed, placed in a centrifuge cooled to 4°C, and centrifuged for 20 minutes at 5000g. When the centrifuge cycle was completed, the tubes were removed, and the supernatant was carefully decanted making sure to not disturb the cell pellet that remained. While decanting, a high flow of N₂/CO₂ (80:20) was introduced into the centrifuge tubes to keep the cell pellet anoxic. After decanting, each cell pellet was resuspended with 1 mL of 10 mM, anaerobic, HEPES buffer. Each 1 mL volume of cell/buffer suspension was distributed into a sterile anaerobic pressure tube, headspace flushed with a high flow of N₂/CO₂ (80:20), and sealed with a thick butyl rubber stopper, crimped, and kept on ice. The suspended cells are anaerobically and aseptically transferred to a sterile solution of 10 mM HEPES buffer and 40 mM AQDS. The cell transfer was 10 percent by volume of the total experimental volume (40 mL). The electron donor used was sodium acetate at a final concentration of 10 mM. The cells were incubated at 30°C for 30 hours to reduce the AQDS to AHQDS, at which time the solution was a dark orange color. Following the reaction, the cells were filtered out of solution using sterile, 0.2μ syringe filters. The AHQDS was transferred into a sterile and anaerobic serum bottle, sealed with a thick butyl rubber stopper, crimped, wrapped in aluminum foil, and kept in the dark.

Abiotic Experiments

Preliminary adsorption experiments were performed using only uranium and either Fe-Gel or γ-Al₂O₃ in solution, in order to determine the optimal equilibration time and mass loading of solids for uranium uptake. Mass loadings evaluated of either γ-Al₂O₃ or Fe-Gel were 2.5 g L⁻¹, 5 g L⁻¹, 10 g L⁻¹, and 20 g L⁻¹. The first set of abiotic reduction experiments were performed similar to the adsorption experiments, except that AHQDS was added to solution and only 5 g/L of each solid was evaluated. The second set of abiotic reduction experiments were similar to the first set, except that 1, 5, and 10 mM carbonate concentrations were added to solution. All experiments were conducted in an anaerobic glove bag under a N₂ atmosphere with H₂ at a background concentration of 3-5 percent. The reactions took place in glass Pyrex bottles with sealable screw caps and were magnetically stirred with Teflon coated magnetic stir bars. Experiments without carbonate were buffered at pH 8 with 10 mM HEPES buffer and HCl or NaOH were added as required to adjust pH. The initial concentration of UO₂Cl₂•3H₂O in all experiments was 100 μM.

Following uranium addition, Pyrex bottles were sealed and allowed to equilibrate for up to 12 hours. For preliminary adsorption experiments, samples were taken at 1 and 12 hours. For abiotic reduction experiments, AHQDS was added to solution after the 12 hour equilibrium period at an initial concentration of 2 mM.

One mL samples were taken from the Pyrex bottle reactors for uranium analysis. The samples were placed in 2 mL plastic centrifuge tubes and centrifuged at 7000g for 5 minutes. After centrifugation, the tubes were removed and the supernatant was carefully decanted, making sure not to disturb the solids pellet, into clean 2 mL centrifuge tubes and stored. The solids remaining in the tube were washed with 1 mL of 10 mM HEPES buffer and resuspended with a Genie Vortex for 5 minutes. The purpose of the washing was to remove and dilute any residual reductant that remained in the solid pores. After re-suspension, the solids were centrifuged at 7000g for 5 minutes. The wash supernatant was decanted, making sure not to disturb the solids pellet, into a clean 2 mL centrifuge tube and stored. To extract the soluble uranium, 1 mL of a solution of $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ (100 mM/ 20 mM) was added to the mineral solids pellet; the pellet was resuspended, and then allowed to equilibrate on the Genie Vortex for 24 hours. Next, the centrifuge tube was centrifuged at 7000g for 5 minutes and the extractant supernatant was carefully decanted and stored. The remaining solids pellet was stored. Each of the three supernatants, i.e., initial, wash, and extractant, were analyzed for U(VI) to verify absorbance and extractable U(VI). Control experiments were performed using AQDS instead of AHQDS, and followed the same protocol. Control experiments were performed using no solids, and also followed the same protocol; however no solid equilibration and sample centrifugation was necessary. Sampling for the solids free control experiments involved taking samples directly from the Pyrex bottles and amending them with the analytical reagent for U(VI) quantification.

Spectrophotometric Quantification of U(VI) by Arsenazo III

Sample analysis and quantification of U(VI) in these experiments was determined spectrophotometrically by Arsenazo III as described previously by Yong et. al. (Yong, Eccles et al. 1996) and Fritz et. al. (Fritz and Bradford 1958). Arsenazo III was prepared by adding 0.15% w/v of dry Arsenazo III to nanopure water. In these experiments, Arsenazo III reagent was prepared in 25 mL stocks, which corresponded to adding 0.375 g of dry Arsenazo III to 25 mL of nanopure water. The solution was mixed well and allowed to sit for one hour. After one hour, the solution was filtered with Whatman filter paper to remove any residual solids. The final stock solution was kept in a glass serum bottle and stored in the dark. Sample analyses were conducted in a 96 well plate spectrophotometer at a

wavelength of 652 nm. Analytical sample volumes were distributed as follows: 14.3 μL 0.15% w/v Arsenazo III, 4.3 μL experimental sample, 281.4 μL 0.1 N HCl.

Results and Discussion

Homogeneous Abiotic Reduction of U(VI) in Solution

Figures 1 and 2 are plots of U(VI) reduction over time in homogeneous systems without and with carbonates, respectively.

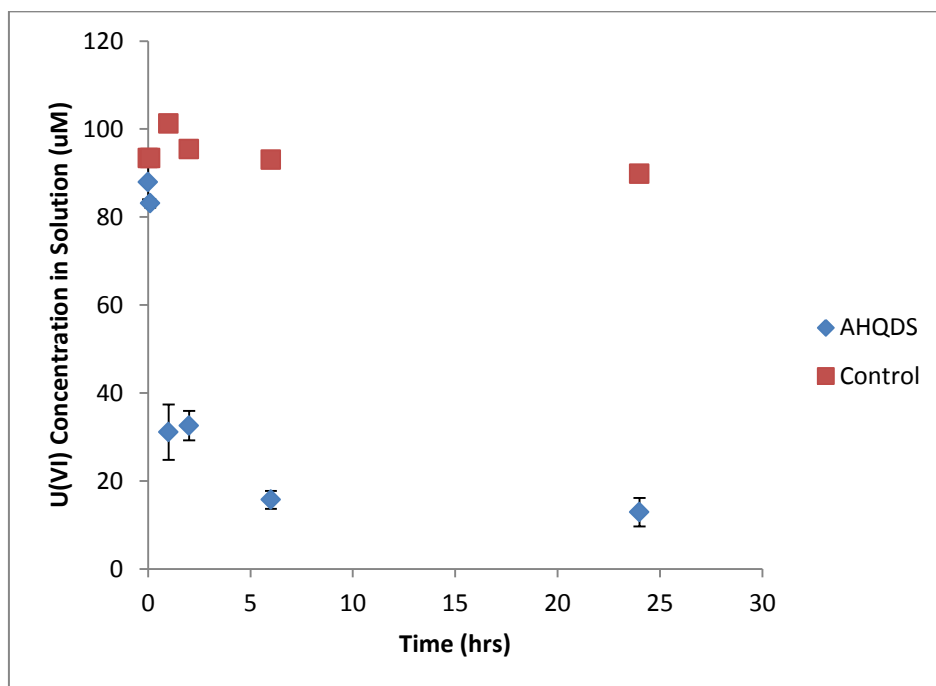


Figure 1 - U(VI) Reduction in a Homogeneous HEPES Buffered System.

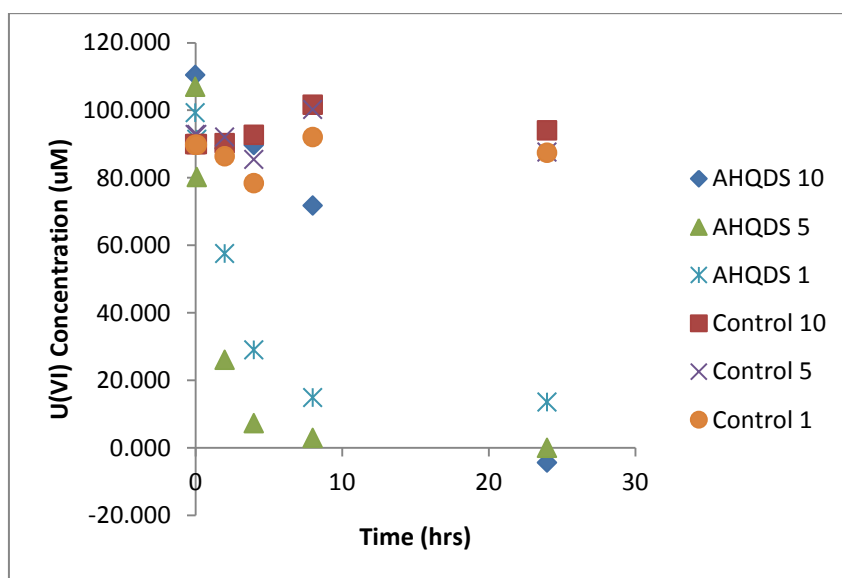


Figure 2 - U(VI) Reduction in a Homogeneous HEPES Buffered System with varying NaHCO_3 Concentrations. The control system is AQDS, and 10, 5, and 1 are respective concentrations (mM) of NaHCO_3 .

It is clearly evident in Figure 1 that U(VI) reduction proceeds rapidly in a homogeneous solution phase in the absence of carbonates. Within 6 hours U(VI) was reduced by AHQDS to 16 μM . This reduction occurred faster than any of the previous experiments discussed in this work. This evidence suggests that U(VI) can be reduced by a bulk reductant in a homogeneous phase, and does not necessarily need to be adsorbed to a surface for reduction to occur.

Figure 2 displays the results for homogeneous reduction experiments in the presence of differing concentrations of carbonates. In these experiments U(VI) was readily reduced by AHQDS as compared to systems where the U(VI) was adsorbed to a solid mineral surface. The presence of carbonates seemed to have an initial inhibition on U(VI) reduction only in the system with 10 mM NaHCO_3 . Comparing this system with those containing 1 mM and 5 mM NaHCO_3 clearly demonstrates this effect. After 8 hours the 1 mM and 5 mM systems have 15 μM and 3 μM of detectable U(VI) in solution, respectively. In contrast, at the same time point, the 10 mM system has 72 μM present. After 24 hours, however, the 10 μM system had no detectable U(VI). These results suggest that overall, carbonates at low concentrations (1 mM and 5 mM), have no inhibiting effect on homogeneous U(VI) reduction by AHQDS. When carbonate concentrations increase (10 mM) an initial stabilizing effect is noticed, however, U(VI) reduction still occurs within 24 hours.

Adsorption and Desorption of Uranium to Mineral Solids

The results of the U(VI) adsorption experiments using $\gamma\text{-Al}_2\text{O}_3$ and Fe-Gel are shown in Figure 3.

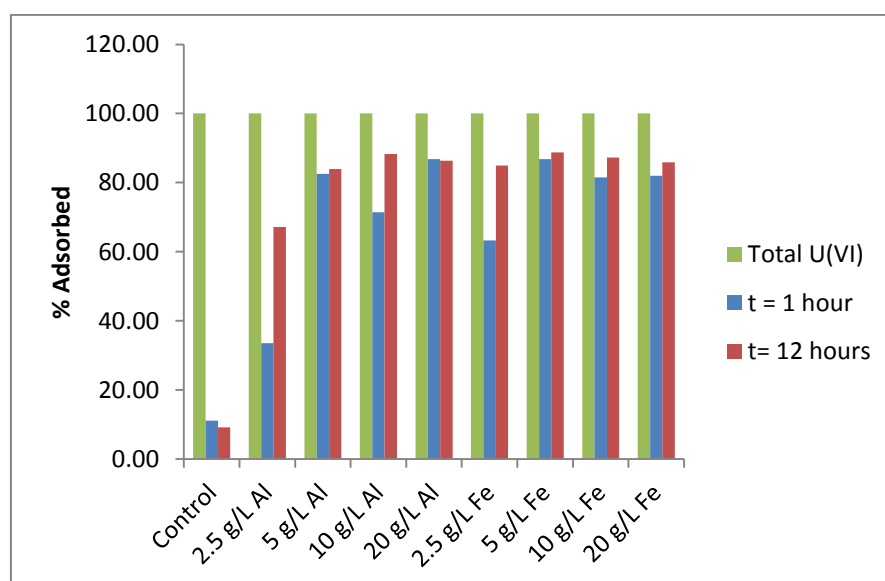


Figure 3 - Adsorption Efficiencies of Uranium to different mass loadings of $\gamma\text{-Al}_2\text{O}_3$ and Fe-Gel.

Comparing the amount of U(VI) adsorbed after one hour and 12 hours shows that, in most systems, the majority of U(VI) adsorption occurs in the first hour. After 12 hours, only 67% of the total U(VI) in the system was adsorbed to the γ -Al₂O₃ surface, whereas after the same equilibration time 85% of the total U(VI) was adsorbed to the Fe-Gel. Also after 12 hours, 84%, 88%, and 86% of the total amended U(VI) was adsorbed to the γ -Al₂O₃ surface at loadings of 5 g L⁻¹, 10 g L⁻¹, and 20 g L⁻¹ respectively. Similarly, 89%, 87%, and 86% of total amended U(VI) was adsorbed to the Fe-Gel surface in the 5 g L⁻¹, 10 g L⁻¹, and 20 g L⁻¹ systems, respectively. Hence, Fe-Gel appears to have more capacity for U(VI) at the low mass loading of 2.5 g/L, but adsorption efficiencies for higher mass loadings are not significantly different from each other. Given the error in U(VI) measurements (\pm 15 μ M), the results are also not significantly different than 100% adsorption efficiency, and this explains why percentages do not increase for loadings in excess of 5 g/L. This agrees with work from Jeon et. al. (Jeon, Dempsey et al. 2005), who showed that over a 20 day equilibration, more than 95% of the U(VI) was adsorbed to goethite and Abbott's Pitt Sand in the first 8 hours of equilibration.

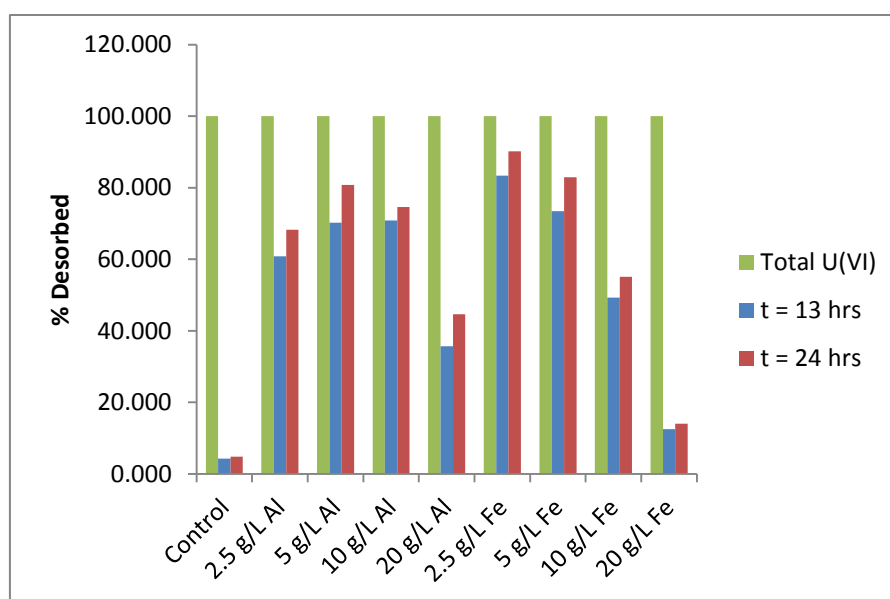


Figure 4 – Desorption Efficiencies of Uranium to different mass loadings of γ -Al₂O₃ and Fe-Gel.

The percent of desorbed uranium after 13 and 24 hours in the presence of 100 mM/20 mM NaHCO₃/Na₂CO₃ (CARB) are shown in Figure 4. Results at 13 and 24 hours are not significantly different based on \pm 15 μ M error of our analytical method. As solid mass loadings increase, U(VI) desorption decreases. For Fe-Gel, the decrease in percent desorbed occurs with each increment of solids added. For γ -Al₂O₃, the percent desorbed is constant

until 20g/L of solid is added, and a relatively sharp decrease is observed. The percent desorption of U(VI) in both systems with 5 g L⁻¹ of solids was 81% and 83% for γ -Al₂O₃ and Fe-Gel, respectively. In subsequent experiments, a mass loading of 5 g L⁻¹ of solids is used, with an adsorption equilibration time of 12 hours and a desorption equilibration time of 12 hours.

Abiotic Reduction of U(VI) on γ -Al₂O₃ in the Absence of Carbonates

The concentration of U(VI) over time in solution after equilibration with γ -Al₂O₃ is shown in Figure 5, both in the presence of AHQDS and AQDS. Less than 15% of the total 100 μ M U(VI) added to the systems is free in solution; thus, 85% of the U(VI) is either adsorbed as U(VI) to the surface of γ -Al₂O₃, or present as insoluble U(IV), regardless of whether AHQDS or AQDS is present.

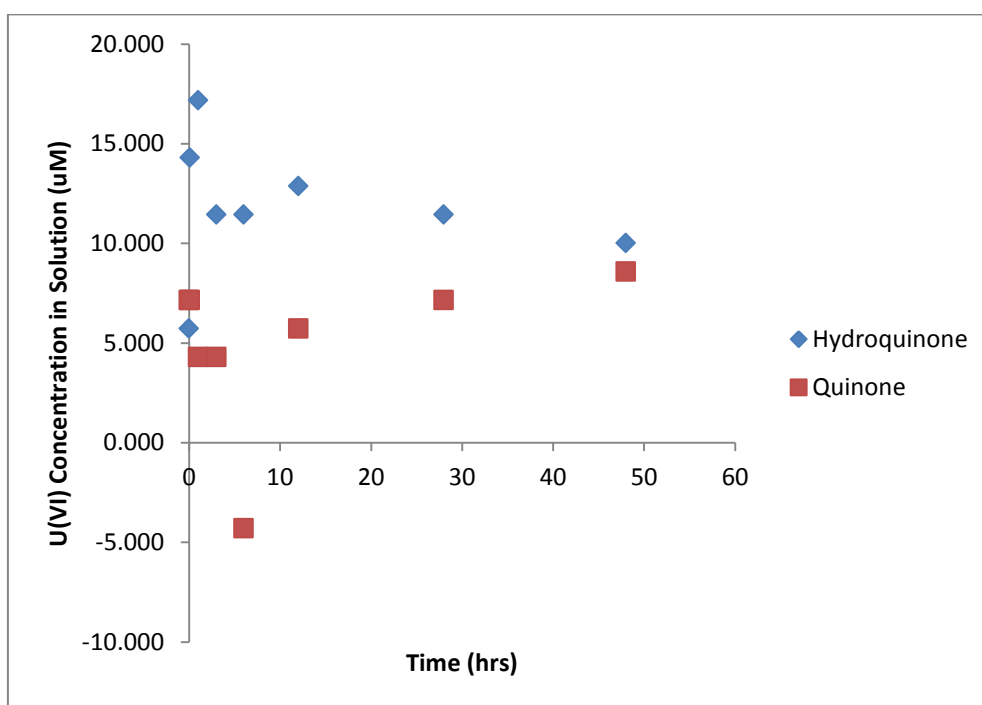


Figure 5 - U(VI) Concentration in Solution over time after adsorption to γ -Al₂O₃ in a carbonate free system.

The concentration of extractable U(VI) over time from the surface of γ -Al₂O₃, as well as the pH values at each time sample, are shown in Figure 6. pH variability over time was small and was +/- 0.6 pH units from pH 8. The U(VI) extracted from the AQDS system relative to the AHQDS system was large, and remained above 70 μ M at all times. This indicates that in the presence of AQDS, amended U(VI) adsorbs to the surface of γ -Al₂O₃ in the form of U(VI). In contrast, the amount of extractable U(VI) in the presence of AHQDS

steadily decreased over time to approximately 6.8% of the original 100 μ M. This suggests that U(VI) is reduced to U(IV) on the γ -Al₂O₃ surface in the presence of AHQDS.

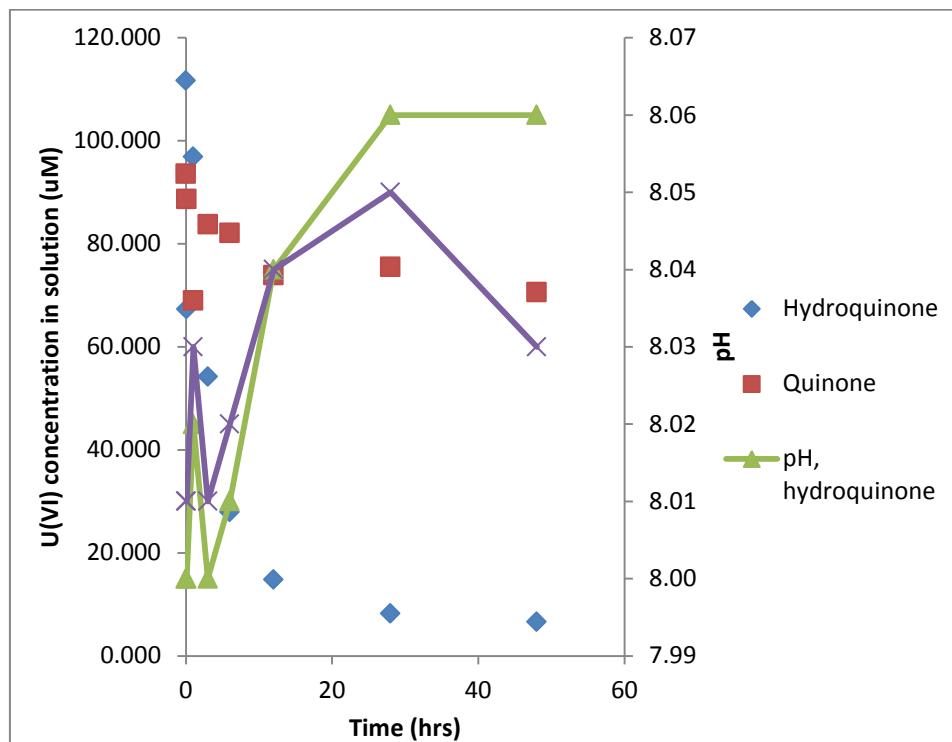


Figure 6 - Extractable U(VI) from the γ -Al₂O₃ surface over time with pH plotted in a carbonate free system.

Abiotic Reduction of U(VI) on γ -Al₂O₃ in the Presence of Carbonates

The concentration of extractable U(VI) from the surface of γ -Al₂O₃ over time, as well as the free U(VI) concentration in solution after equilibration in the presence of carbonates, is shown in Figure 7. By examining the data of extractable U(VI) and free U(VI), the results show that, on average, more than 90% of the total U(VI) added in each system remained adsorbed to the surface of γ -Al₂O₃ throughout the course of the experiment. Comparing figure 6 and figure 7, there is a large increase in time to reduce adsorbed U(VI). This result agrees with our hypothesis that the presence of carbonates in a system will have a stabilizing effect on the U(VI), thus inhibiting reduction.

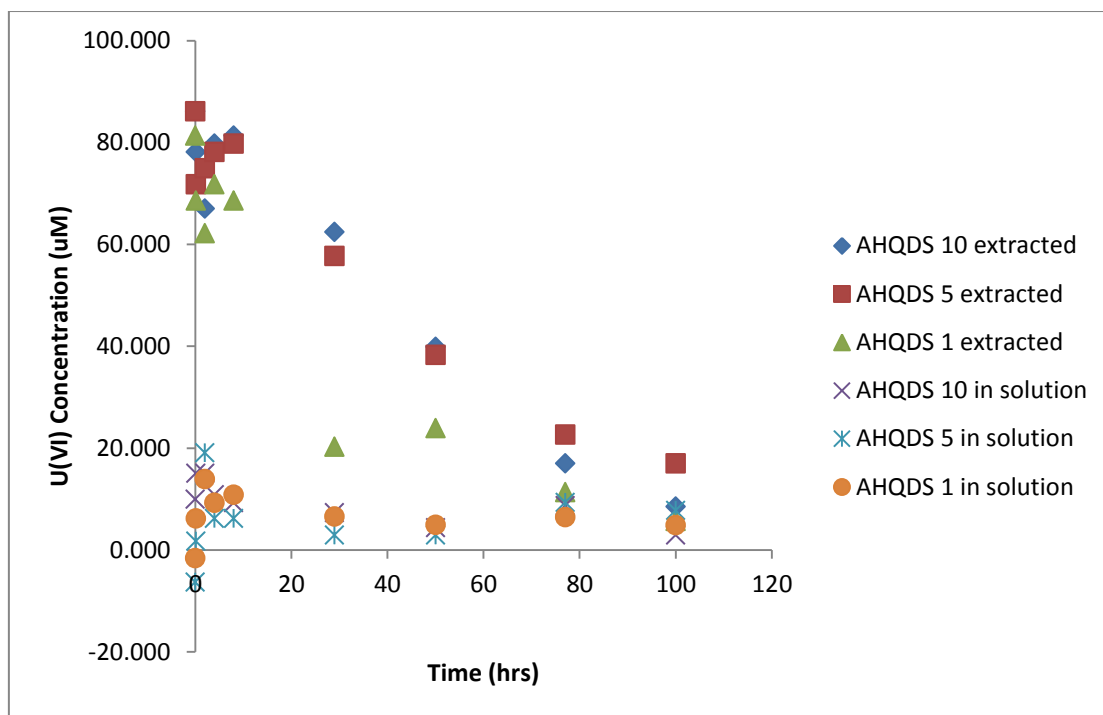


Figure 7 - Concentrations of extractable U(VI) and U(VI) in solution over time in a pH 8 HEPES buffered system with $5 \text{ g L}^{-1} \gamma\text{-Al}_2\text{O}_3$ solids in a carbonate system. The 10, 5, and 1 in the legend are representative of the concentration (mM) of carbonates in the system.

The first 50 hours of figure 7 are shown in figure 8 with preliminary kinetic data. The first 50 hours were chosen because most reduction of U(VI) occurs within this time period. The 5 mM and 10 mM NaHCO_3 systems follow a zero order reaction rate with corresponding rates of $-0.8132 \mu\text{mol L}^{-1} \text{h}^{-1}$ and $-0.7608 \mu\text{mol L}^{-1} \text{h}^{-1}$, respectively. The system containing 1 mM NaHCO_3 approached detection limits in 29 hours, as compared to 100 hours and 77 hours for the 5 mM and 10 mM NaHCO_3 systems, respectively. The reduction rate of the 1mM NaHCO_3 was significantly faster at $-1.1544 \mu\text{mol L}^{-1} \text{h}^{-1}$. These results support the hypothesis that in the presence of carbonates, U(VI) reduction is inhibited. The stabilizing effect could be due to the carbonate-U(VI) coordination complex inhibiting electron transfer due to ligand steric hindrance effects.

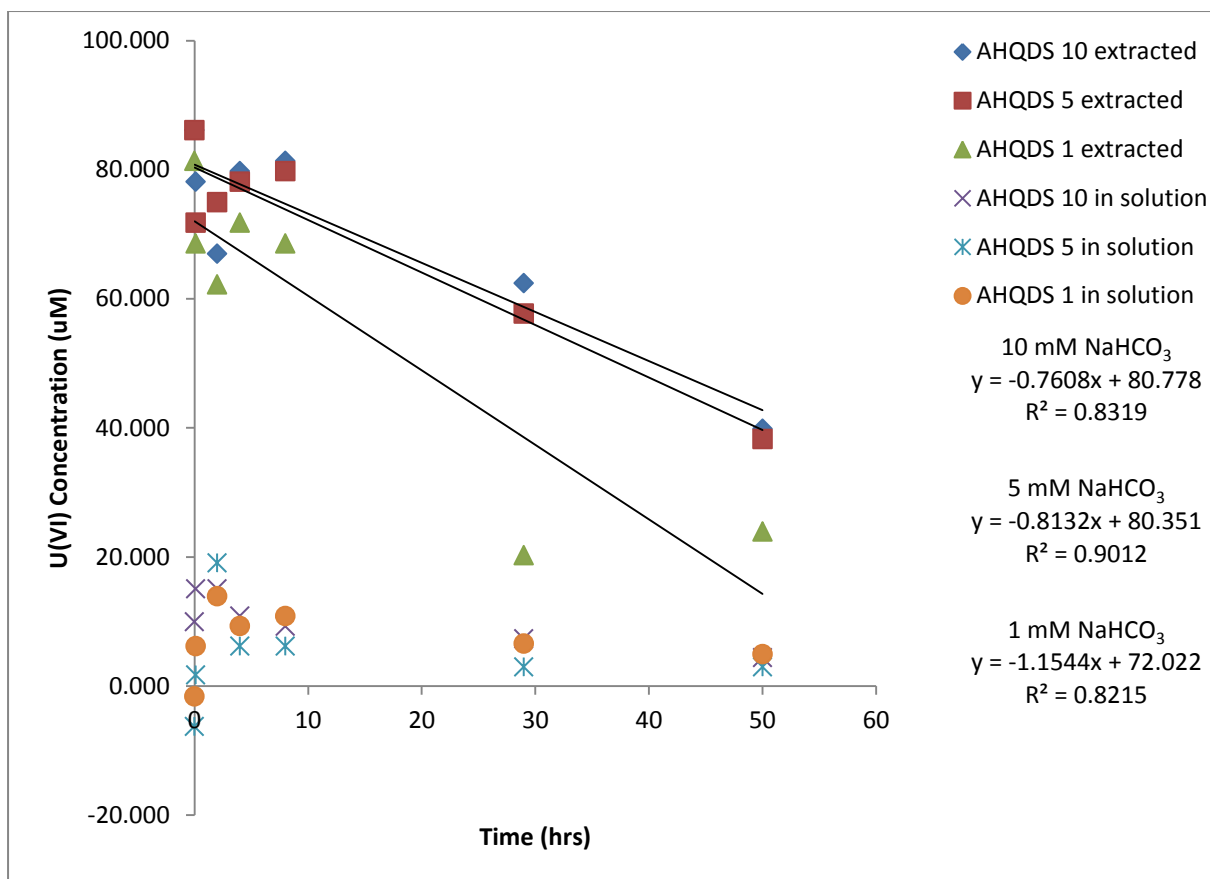


Figure 8 - U(VI) Reduction Rate Analysis up to 50 hours in a pH 8 HEPES buffered system with 5 g L⁻¹ γ -Al₂O₃ solids in a carbonate system. The 10, 5, and 1 in the legend are representative to the concentration (mM) of carbonates in the system.

Abiotic Reduction of U(VI) on Fe-Gel in the Absence of Carbonates

The concentration of U(VI) over time in solution after equilibration with Fe-Gel and either AQDS or AHQDS is shown in Figure 9. Less than 10% of the total 100 μ M U(VI) added to the systems is free in solution. Therefore, more than 90% of the total 100 μ M U(VI) amended to the systems is adsorbed to the surface throughout the course of the experiment, regardless of whether AQDS or AHQDS is present. The concentration of extractable U(VI) over time from the surface of FeOOH in the presence of either AQDS or AHQDS, as well as the pH values at each time sample, are shown in Figure 10. The pH variability is small, and within +/- 0.18 units.

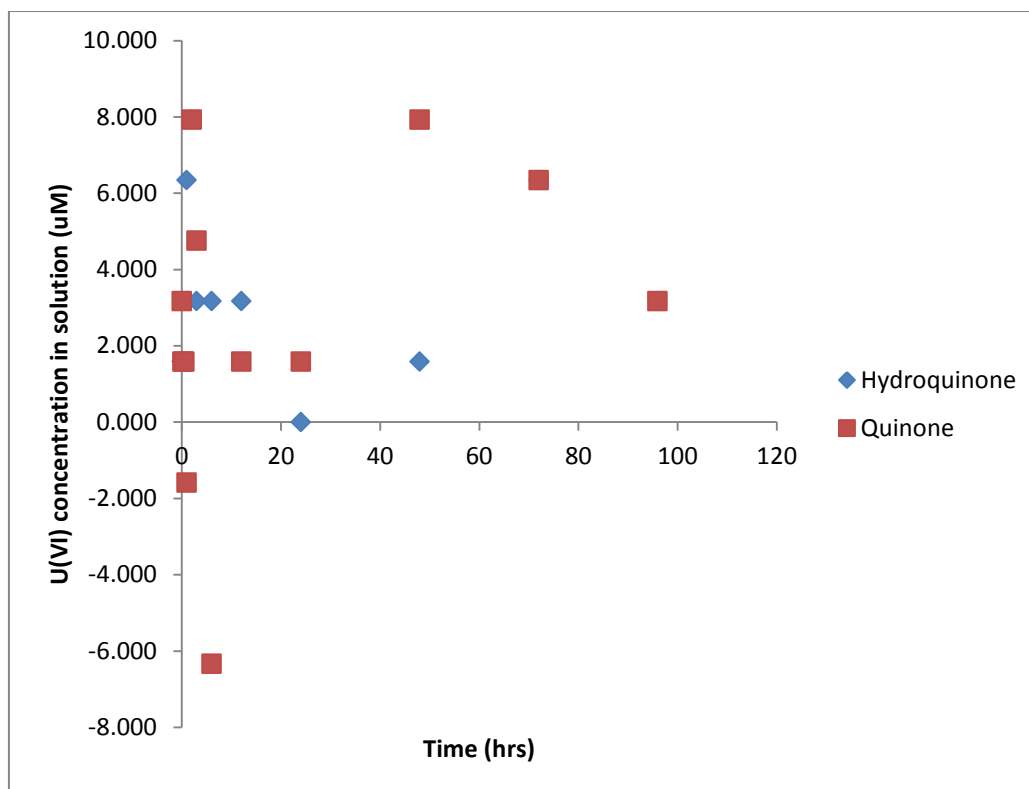


Figure 9 – U(VI) Concentration in Solution over time after adsorption to Fe-Gel in a carbonate free system.

The amount of U(VI) extracted decreased in the presence of either AQDS or AHQDS, but more so in the presence of the former. In the presence of AQDS, approximately 56% of the initial U(VI) was extracted from the solids, whereas in the presence of AHQDS, only 10.9% of the initial U(VI) was extracted. This suggests that U(VI) is reduced to U(IV) on the Fe-Gel surface in the presence of AHQDS. The results agree with our hypothesis that U(VI) reduction would occur while adsorbed to a redox-active mineral surface.

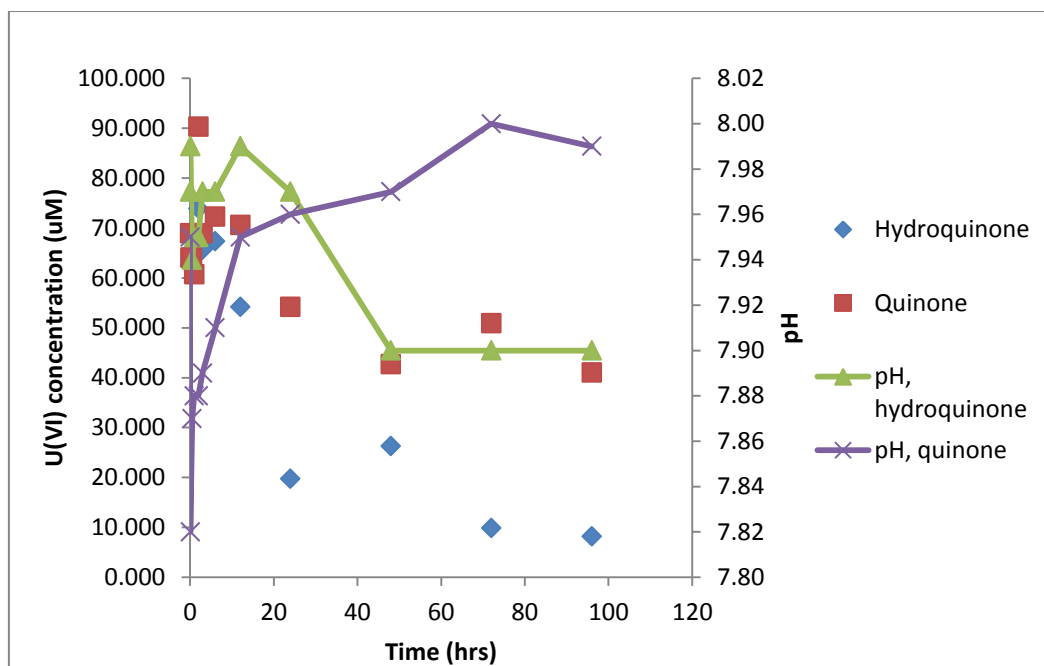


Figure 10 - Extractable U(VI) from the Fe-Gel surface over time with pH plotted in a carbonate free system.

Comparing the Fe-Gel system to the $\gamma\text{-Al}_2\text{O}_3$ system (Figures 10 and 6), respectively, the time for extracted U(VI) to reach the detection limit of 15 μM in the former was about twice the latter, indicating reduction on the former lags reduction on the latter. This could be due to competition for AHQDS by U(VI) and FeOOH solids. The latter is known to accept electrons to reduce Fe(III) to Fe(II). Colorimetric observation supports this assertion. AHQDS is a dark orange color, while AQDS is a very pale yellow color. In the FeOOH experiments, AHQDS changes from orange to yellow in 5 minutes, while in the $\gamma\text{-Al}_2\text{O}_3$ experiments, the orange color persists at all time points. These results are contrary to the hypothesis that the redox active FeOOH will enhance U(VI) reduction relative to $\gamma\text{-Al}_2\text{O}_3$.

Abiotic Reduction of U(VI) on Fe-Gel in the Presence of Carbonates

The concentration of extractable U(VI) from the surface of Fe-Gel over time, as well as the free U(VI) concentration in solution after equilibration in the presence of carbonates, is shown in Figure 11. Comparing figure 10 and figure 11, there is a noticeable

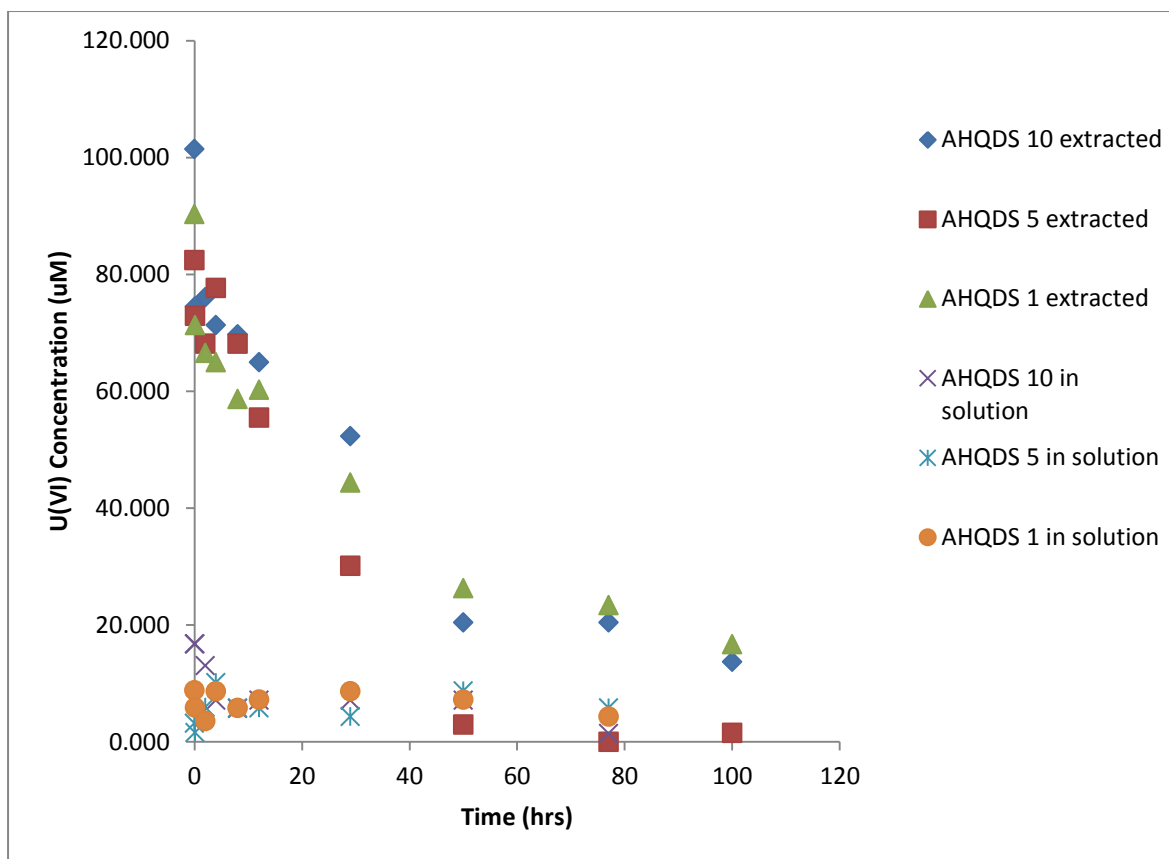


Figure 11 - Concentrations of extractable U(VI) and U(VI) in solution over time in a pH 8 HEPES buffered system with 5 g L⁻¹ Fe-Gel solids in a carbonate system. The 10, 5, and 1 in the legend are representative of the concentration (mM) of carbonates in the system.

increase in time to reduce adsorbed U(VI). The time to reduce U(VI) to detection limits in the carbonate system increased by at least 20 hours compared to the carbonate free system. This result agrees with our hypothesis that the presence of carbonates in a system will have a stabilizing effect on the U(VI), thus inhibiting reduction.

The results of this experiment demonstrate that reduction rates are similar across all systems containing varying concentrations of carbonate. This initial data suggests that NaHCO₃ concentrations above 1 mM do not appear to have any significant effect on the reduction of U(VI) adsorbed to Fe-Gel. Thus, in the presence of carbonates, reduction rates are slowed, but reduction rates are similar across different carbonate concentrations. These results leave an open question on why increasing carbonate concentrations did not affect the results on a redox-active surface. When comparing γ -Al₂O₃ and Fe-Gel abiotic reduction experiments with carbonates present, figures 7 and 11, respectively, the Fe-Gel system with 5 mM NaHCO₃ and the γ -Al₂O₃ system with 1 mM NaHCO₃ reduced U(VI) within 48 hours. When further comparing figures 7 and 11, it is evident that all experimental systems with

Fe-Gel reached an extractable U(VI) concentration of 25 μM or less within 48 hours. With the exception of the 1 mM $\gamma\text{-Al}_2\text{O}_3$ NaHCO_3 system, the 5 mM and 10 mM NaHCO_3 $\gamma\text{-Al}_2\text{O}_3$ systems reached similar concentrations within 77 hours. The difference in time suggests that a surface catalyzed reduction of adsorbed U(VI) is kinetically favored in the presence of carbonates, whereas the direct reduction of adsorbed U(VI) by a bulk reductant is not as favorable in the presence of carbonates. That is, the transfer of electrons from the Fe(II) surface to the adsorbed U(VI) is kinetically favored over the electron transfer from the hydroquinone in solution to the adsorbed U(VI) in the presence of carbonates.

The first 50 hours of figure 11 are shown in figure 12 with preliminary kinetic data. The first 50 hours were chosen because most reduction of U(VI) occurs within this time period. The 5 mM and 10 mM NaHCO_3 systems have corresponding reduction rates of $-1.5216 \mu\text{mol L h}^{-1}$ and $-1.2118 \mu\text{mol L h}^{-1}$, respectively. Using the same analysis the system containing 1 mM NaHCO_3 had a slower reduction rate of $-0.982 \mu\text{mol L h}^{-1}$. These results could suggest that while U(VI) is adsorbed on a redox-active mineral surface, the concentration of carbonates in a system will have a minor influence on the overall reduction rate of the adsorbed U(VI). This can further suggest that in a naturally buffered system, while U(VI) is adsorbed on a redox-active surface, it predominantly undergoes a surface catalyzed reduction reaction that is not influenced by the presence of carbonates. The reduction rates realized on Fe-Gel (Figure 12) are significantly faster than those on $\gamma\text{-Al}_2\text{O}_3$ (Figure 8). This result suggests that in the presence of a carbonate buffered system, surface catalytic reduction of adsorbed U(VI) is favorable to direct reduction of adsorbed U(VI) via solution phase AHQDS. As described in the previous sections, color observation supports this due to the absence of the characteristic orange color of AHQDS minutes after initial amendment.

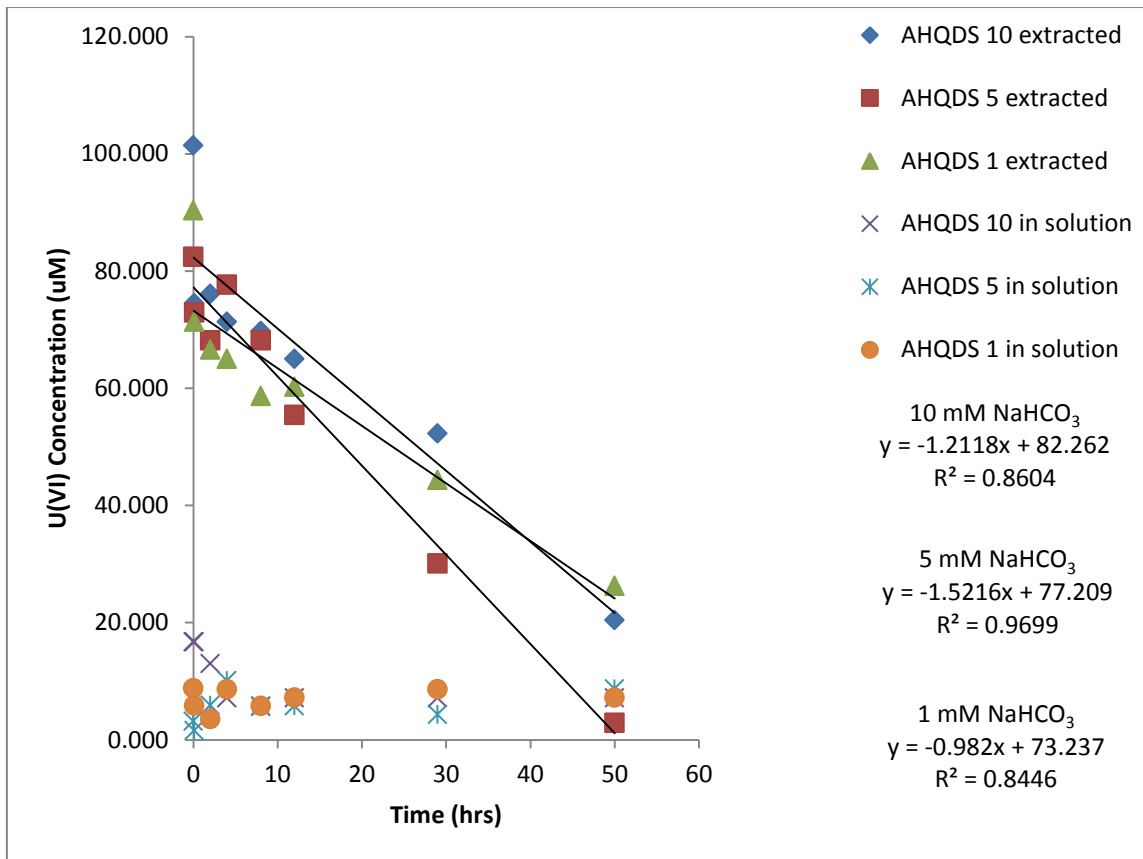


Figure 12 - U(VI) Reduction Rate Analysis up to 50 hours in a pH 8 HEPES buffered system with 5 g L⁻¹ Fe-Gel solids in a carbonate system. The 10, 5, and 1 in the legend are representative to the concentration (mM) of carbonates in the system.

Conclusion

The experiments presented in this work are preliminary studies investigating the roles of redox-active and non-redox-active mineral surfaces on the abiotic reduction of adsorbed U(VI) by extra-cellular electron shuttles such as AHQDS. Additionally, the role of carbonates on U(VI) reduction were also studied. The main hypothesis tested were 1) Redox-active mineral surfaces are responsible for reduction of adsorbed U(VI), and 2) The presence of carbonates in a system will inhibit U(VI) reduction.

As the results show, the first hypothesis was disproven by the results shown in the experiments with γ -Al₂O₃. Overall, however, the reduction rates in the Fe-Gel system were higher than those in the γ -Al₂O₃ system. This could suggest that redox-active mineral surfaces do play a role in enhancing reduction via a surface catalysis effect. Hypothesis one was further disproven by the homogeneous control experiments. In these experiments reduction was realized rapidly in both the absence and presence of bicarbonates. This suggests that electron transfer can occur efficiently and directly in solution from AHQDS to solvated U(VI). The second hypothesis is supported as suggested by the data in the systems containing mineral solids. In a homogeneous system, though not conclusive, the 10 mM system shows evidence of reduction inhibition.

As stated previously, these experiments are preliminary works to study the influence mineral surfaces and carbonates have on U(VI) reduction. Though the results presented in this work are by far conclusive, the results suggest evidence that electron transfer happens directly from electron shuttles to metal ions regardless of whether the metal is adsorbed to a solid or is free in solution, and carbonates inhibit the reduction of the metal, primarily when adsorbed on a surface. Future work must be conducted to investigate these mechanisms to obtain conclusive evidence that supports what is suggested in this work.

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Appendix A

The work contained in appendix A is a preliminary study to attempt the sustained growth of *Geobacter metallireducens* (GS-15) in a microfluidic pore network utilizing uranyl chloride as the terminal electron acceptor. Due to the strong complexation of uranyl with inorganic phosphorus, a modified media using an organic phosphorus source was used to prevent precipitation. All experiments utilized pure cultures grown in ferric citrate media and were transferred at a ratio of 0.03 mL/mL (pure culture/experiment volume). The uranium growth experiments were conducted in fresh water media at 30°C, in anaerobic pressure tubes, under a N₂/CO₂ (80:20) atmosphere. The anaerobic pressure tubes were sealed with thick butyl rubber stoppers and crimped. The freshwater media was prepared with the following components (g/L unless specified otherwise): NaHCO₃, 2.5; NH₄Cl, 0.25; modified Wolfe's vitamin and mineral mixtures (each 10 mL/L) from stocks and 1 mL of 1 mM Na₂SeO₄. The final concentrations of vitamins in the freshwater media are as follows: 20 µg biotin, 20 µg folic acid, 100 µg pyridoxine HCl, 50 µg riboflavin, 50 µg thiamine, 50 µg nicotinic acid, 50 µg pantothenic, 1 µg B-12, 50 µg p-aminobenzoic acid, 50 µg thioctic acid; the final mineral concentrations are: 15 mg NTA, 30 mg MgSO₄, 5 mg MnSO₄•H₂O, 10 mg NaCl, 1 mg FeSO₄•7H₂O, 1 mg CaCl₂•2H₂O, 1 mg CoCl₂•6H₂O, 1.3 mg ZnCl₂, 100 µg CuSO₄•5H₂O, 100 µg AlK(SO₄)₂•12H₂O, 100 µg H₃BO₃, 250 µg Na₂MoO₄, 240 µg NiCl₂•6H₂O, 250 µg Na₂WO₄•2H₂O and 189 µg Na₂SeO₄. Following media preparation and sealing of the pressure tubes, the tubes were autoclaved for 20 minutes at 120°C. Uranium stock was prepared from UO₂Cl₂•3H₂O, stored in an anaerobic serum bottle under an N₂ atmosphere, sealed with a thick butyl rubber stopper, crimped, and autoclaved for 20 minutes at 120°C. Uranium stock was amended to the freshwater media via aseptic and anaerobic techniques to a final concentration of 500 µM. Sodium acetate was the electron donor used for these experiments. Sodium acetate was amended to the experimental series from sodium acetate stock via aseptic and anaerobic techniques to a final concentration of 1 mM or 10 mM depending on the system (described in results). A sterile, anaerobic stock of sodium β-glycerophosphate was prepared and used as the phosphorus source. The sodium β-glycerophosphate stock could not be autoclaved as this releases the inorganic phosphorus into solution. Sterility was achieved by autoclaving a sealed serum bottle containing a N₂ atmosphere at 120°C for 20 minutes. Once the bottle cooled, the sodium

β -glycerophosphate solution was injected into the sealed bottle via aseptic and anaerobic techniques passing the solution through a 0.2 μm filter. The final phosphorus concentration amended into the experimental tubes was 4.35 mM. Samples were taken anaerobically and aseptically and filtered with 0.2 μm syringe filters. The samples were diluted and stored in 0.1 N HCl.

The results shown below show a series of growth studies using U(VI) as the terminal electron acceptor for microbial respiration, utilizing GS-15 as a microbial catalyst. The results compare the absence of U(VI) in solution over time in growth media containing organic or inorganic phosphorus sources. For both the inorganic and organic phosphorus experiments, Sample 1, 2, 3, and 4 follow the same experimental regime. Sample 1 contains 500 μM U(VI), 1 mM acetate, GS-15; Sample 2 500 μM U(VI), 10 mM acetate, GS-15; Sample 3 500 μM U(VI), 0 mM acetate, GS-15; Sample 4 500 μM U(VI), 1 mM acetate, no GS-15.

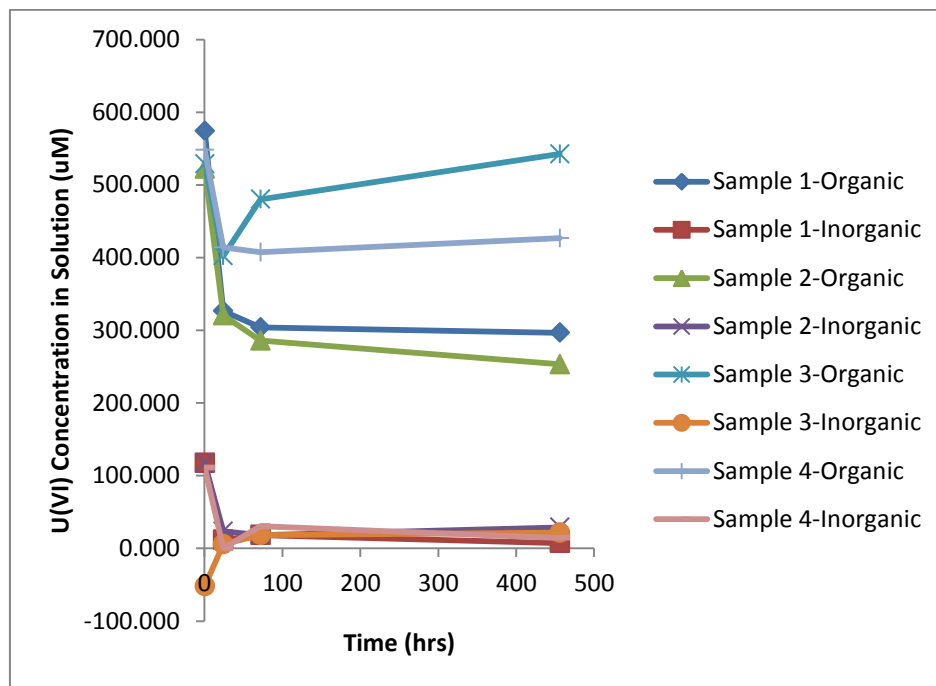


Figure 13 – GS-15 growth studies in Freshwater media with 500 μM uranyl chloride with organic or inorganic phosphorus sources.

The results show a rapid decrease in U(VI) concentration in solution for all experimental systems containing inorganic phosphorus. This is expected since U(VI) rapidly complexes with dissolved phosphate to form insoluble U(VI)-phosphate precipitates. The organic phosphorus systems are inconclusive at this time. All system show initial removal of U(VI) in solution. As time progresses, the system without cells shows a steady increase back to

the initial concentration of U(VI) which could suggest a strong complexation effect with the organic phosphorus. It is important to note that no precipitates were noticed in any of the organic systems, suggesting that the organic phosphorus could chelate the U(VI). The systems with electron donors and cells show initial removal of U(VI) in solution; however the removal stalls after 100 hours. The absence of precipitates could suggest that if the bacteria are in fact reducing U(VI) to U(IV), the organic phosphorus may chelate the U(IV) and prevent it from precipitating.

Further studies need to investigate the growth of GS-15 utilizing U(VI) and an organic phosphorus source to draw conclusive evidence on whether U(VI) reduction is actually occurring, or if the organic phosphorus is interfering with respiration. A second possibility could be that the analytical technique used in this study was not well suited for studying organocomplexes of U(VI).